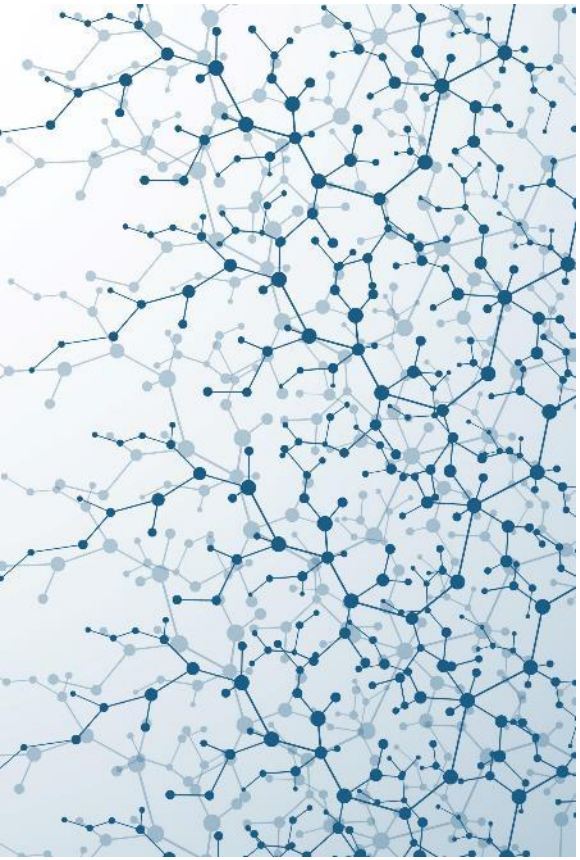
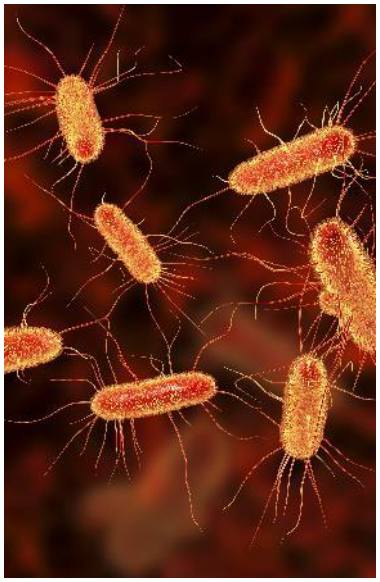
Investigating Effective Alternatives to Silver-based Pressure Sore Treatments and Other Novel Applications of Gold and Copper Nanoparticles





Arjun Ghosh
Los Alamitos High School

Image source. Adobe Stock Photo

Abstract

Recently after undergoing a major surgery, my grandfather developed a pressure sore on his lower back (tailbone) that dug deep into his skin to the point that the bone was visible. To help treat the pressure sore, he was prescribed a silver nanoparticle-based medication. Because silver nanoparticles are known to have cytotoxic and genotoxic side effects, I wanted to find an effective alternative. I found that nanoparticles such as Gold (AuNPs) and Copper (CuNPs) have antibacterial properties and are biocompatible. My project looks to address the problem of silver in the medical field by testing the effectiveness of AuNPs or CuNPs as an alternative. Through my research, I propose to use metal nanoparticles (AuNP and CuNP) with Agar gel to replace the use of silver and establish the effectiveness of the compound. Some other practical applications of metal nanoparticles in antibacterial treatments are also explored. I tested on Escherichia coli (E.coli), the gram-negative bacteria, and Bacillus subtilis (B. subtilis), the gram-positive bacteria. This first phase of my project primarily focuses on using nano-materials in treating bacterial infections in wounds therefore assisting the healing process. Through my project experiments, I concluded gold nanoparticles (AuNP) either did not reach Minimum Inhibitory Concentrations (MIC) for E.coli and B. subtilis or were too inert to be effective. Therefore, I concluded that gold will be ineffective in combating bacteria that forms on pressure sores. Copper demonstrated bactericidal properties by application as a gel, application as wound dressing, and direct application in a suspension.

Problem

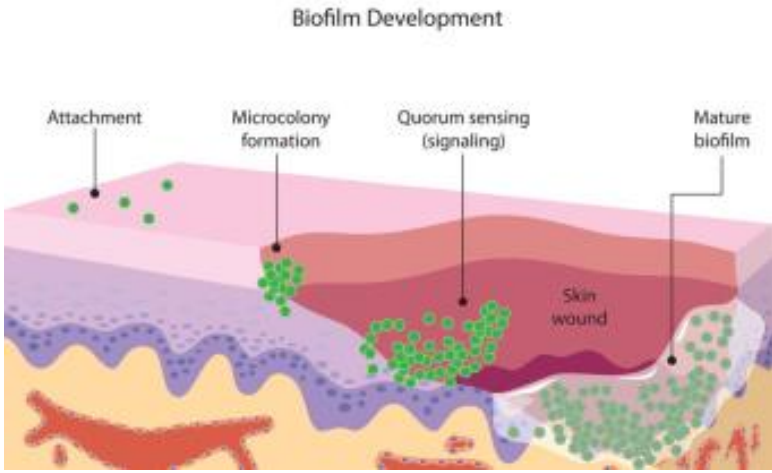
- Silver Nanoparticles (AgNPs) have gained considerable interest in wound infection reduction, as they can release Ag+ ions at a greater rate than bulk silver, by virtue of their large surface area.
  - The control of infections in open wounds present a significant clinical challenge and a multibillion-dollar industry worldwide; an estimated $25 billion spent in the US annually (Sen et al., 2010).
  - Silver has been recognized for centuries as a useful antimicrobial agent. Silver compounds, such as silver nitrate or silver sulfadiazine, are used for topical applications in which the silver ion Ag+ is the active antimicrobial entity.

- However, silver-based treatments have cytotoxic effects on tissues, can cause delayed healing, and have growing concerns of development of antimicrobial resistance (FDA study, 2016).

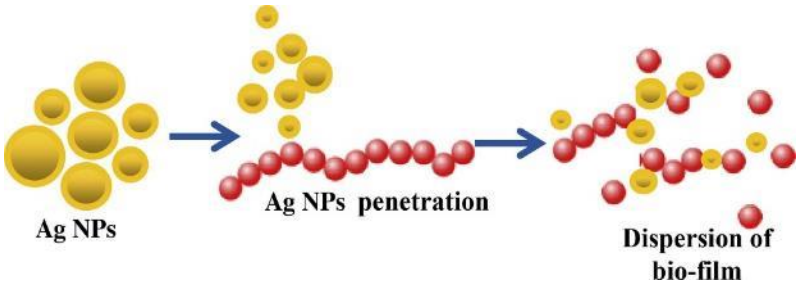
- Gold Nanoparticles (AuNPs) and Copper Nanoparticles (CuNPs) have significant potential to replace the Silver based wound healing process.

Introduction (Background Research)

- The greatest barrier to healing of wounds is biofilm, a colony of bacteria within a matrix of self-produced extracellular polymeric substance.
- Silver Nanoparticles are effective due to their large surface area, small size allowing to penetrate cell wall, release of Ag+ ions at a large rate to neutralize negatively (-) charged bacteria, attacking biofilm.
- AuNPs are widely used as targeted drug delivery systems and is less reactive but, more expensive.
- Cu is an essential micronutrient to the body, its cytotoxicity is less than Ag (Ostaszewska et al., 2018) and has shown to be more antimicrobial than Ag.
- AuNPs and CuNPs have similar mechanisms like AgNP of attacking biofilms.



https://www.woundsource.com/



https://www.sciencedirect.com/

Hypothesis

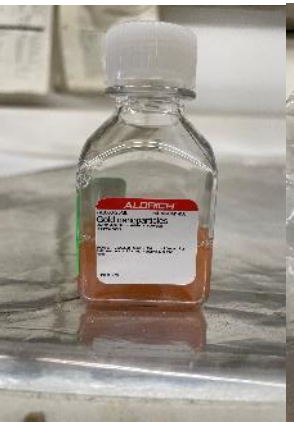
1. My hypothesis is that Gold Nanoparticles (AuNPs) and Copper Nanoparticles (CuNPs) will be effective to combat bacteria that forms on pressure sores, can be a replacement to silver-based treatments, and assist in safe healing of wounds.

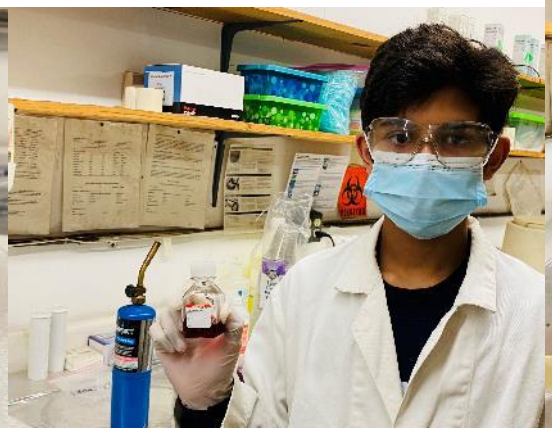
2. My primary objective of this research is to develop an effective and safe antibacterial gel using Gold Nanoparticles (AuNPs) and Copper Nanoparticles (CuNPs).

3. The secondary objective is to find broader applications of Gold and Copper Nanoparticles in antimicrobial treatment in the healthcare industry.

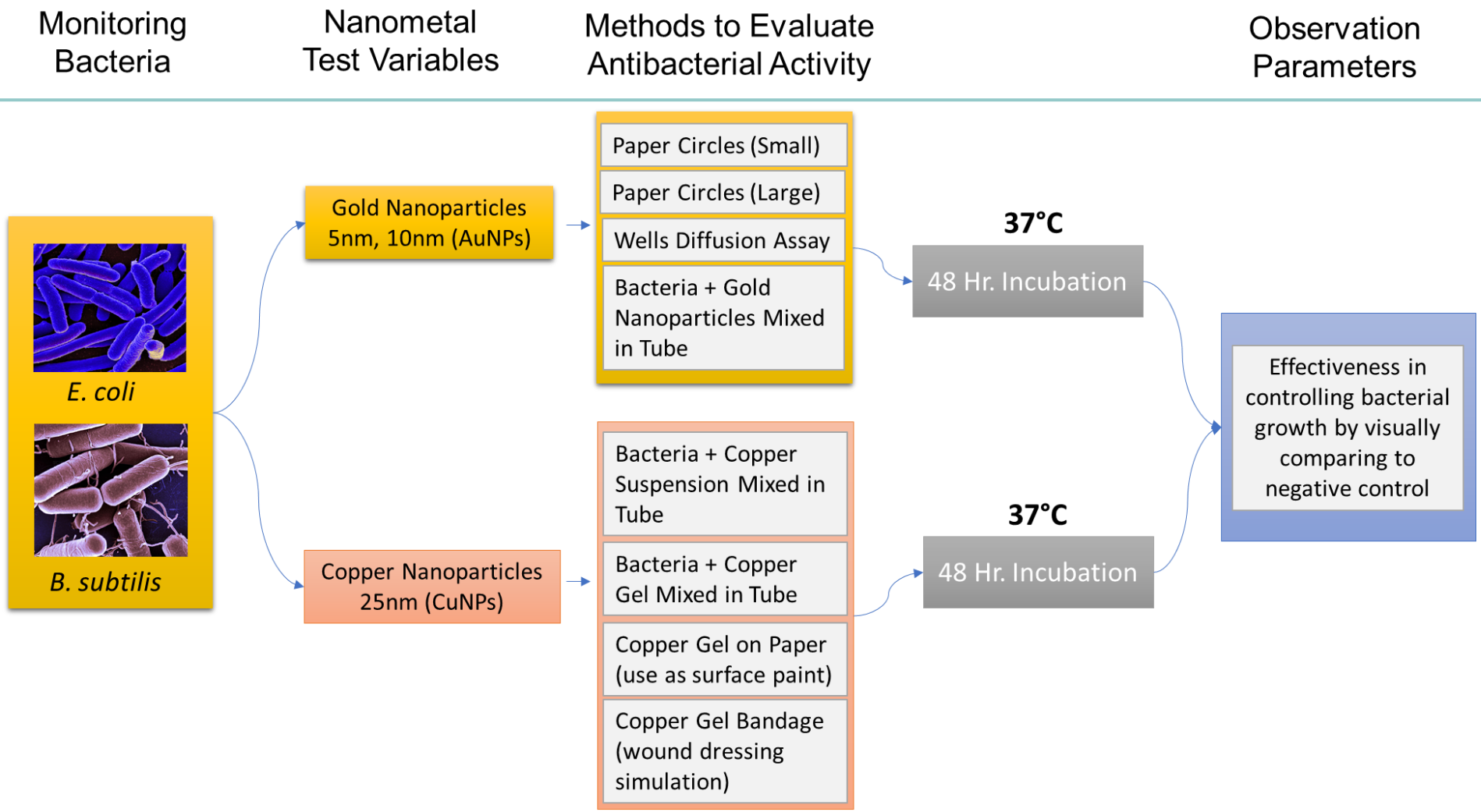
Materials

- Gold Nanoparticles 5nm diameter (Sigma-Aldrich 741949)
- Gold Nanoparticles 10nm diameter (Sigma-Aldrich 741957)
- Copper Nanopowder 25nm diameter (Sigma-Aldrich 774081)
- Escherichia coli (E. coli) (ATCC 8739 LOT 483-1051-82022-05-310483U)
- Bacillus subtilis (B. subtilis) (ATCC 19659 LOT 540-21-22021-10-310540U)
- Tryptic Soy Agar Plates (Hardy Diagnostics G60 LOT 474621)
- Tryptic Soy Broth Tubes (Hardy Diagnostics K82 LOT 475069)
- Chloramphenicol
- Calcium Alginate Swabs, Glass Tubes,
  Deionized water, Alcohol, Agar Powder
- Incubator, Autoclave, Metal loop



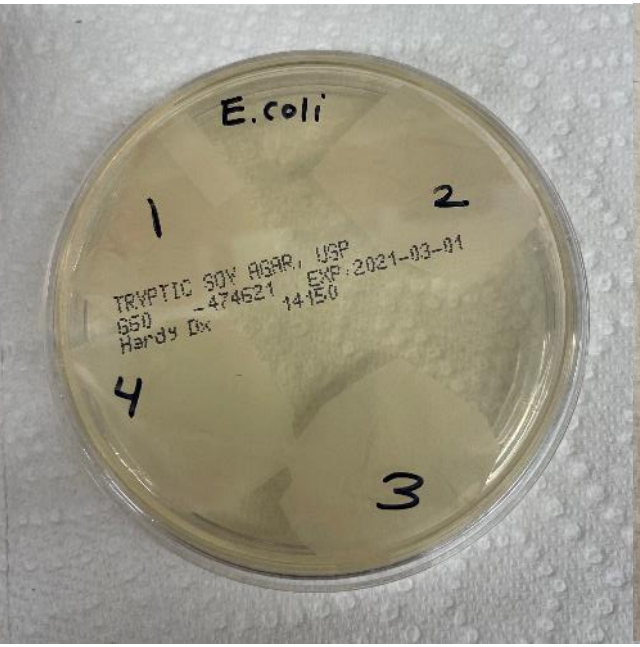


Procedure


Monitoring Bacteria
Nanometal Test Variables
Methods to Evaluate Antibacterial Activity
Observation Parameters
E. coli
B. subtilis
Gold Nanoparticles 5nm, 10nm (AuNPs)
Copper Nanoparticles 25nm (CuNPs)
Paper Circles (Small)
Paper Circles (Large)
Wells Diffusion Assay
Bacteria + Gold Nanoparticles Mixed in Tube
Bacteria + Copper Suspension Mixed in Tube
Bacteria + Copper Gel Mixed in Tube
Copper Gel on Paper (use as surface paint)
Copper Gel Bandage (wound dressing simulation)
37°C
48 Hr. Incubation
37°C
48 Hr. Incubation
Effectiveness in controlling bacterial growth by visually comparing to negative control

Procedure (Continued)

- Multiple methods used to test antimicrobial activities of Gold and Copper NPs
  a) Paper Circles
  b) Wells Diffusion Assay
  c) Mixing NPs with Bacteria before plating



a)

b)

c)

Results

Gold Nanoparticle (AuNP) Results Tables

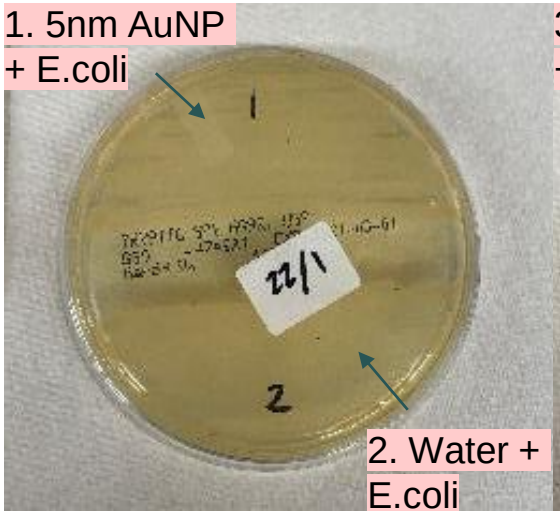
AuNP 5nm					
	50µL	75µL	100µL	200µL	250µL
Weight g/µL	3.4198E-12	5.13E-12	6.83951E-12	1.37E-11	1.71E-11
# of Particles	2.75E+12	4.125E+12	5.5E+12	1.1E+13	1.38E+13
E.coli	* * *	* * *	* * *	* * *	* * *
B. subtilis	* * *	* * *	* * *	* * *	* * *

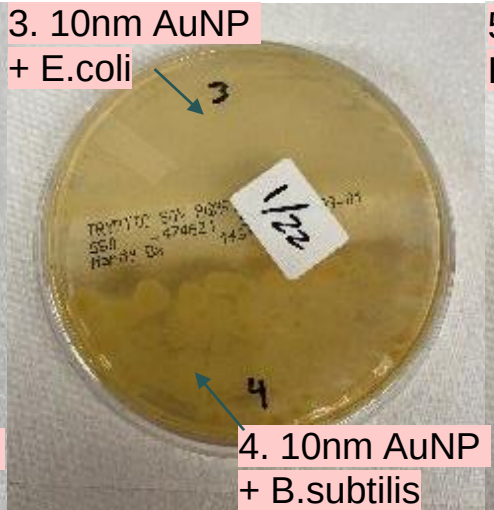
AuNP 10nm					
	50µL	75µL	100µL	200µL	250µL
Weight g/µL	2.9845E-12	4.477E-12	5.96903E-12	1.19E-11	1.49E-11
# of Particles	3E+11	4.5E+11	6E+11	1.2E+12	1.5E+12
E.coli	* * *	* * *	* * *	* * *	* * *
B. subtilis	* * *	* * *	* * *	* * *	* * *

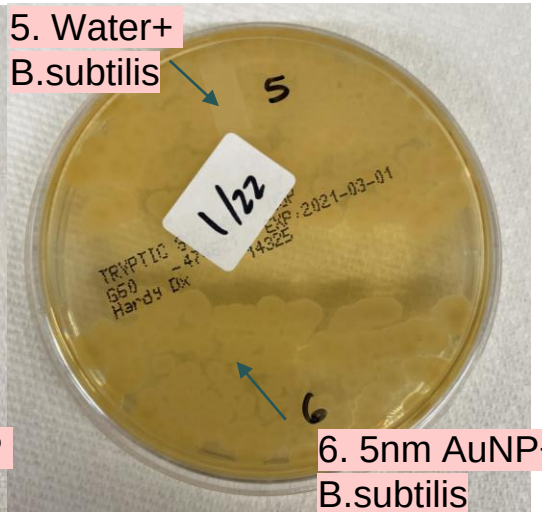
Legend

No bacterial growth	*Less bacterial growth (than negative control)*	*Full bacterial growth (in comparison to negative control)*
*	**	***

Gold Nanoparticle (AuNP) Experiment Results







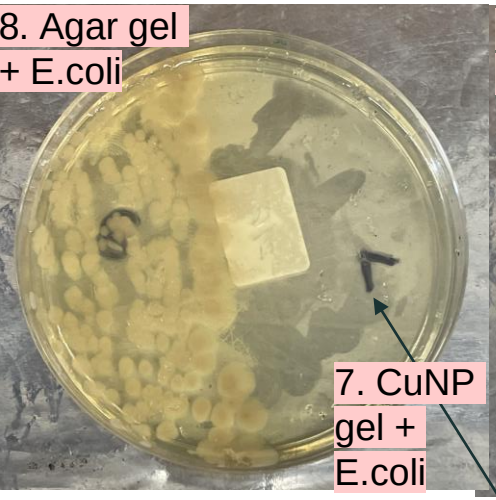
Results (Contin.)

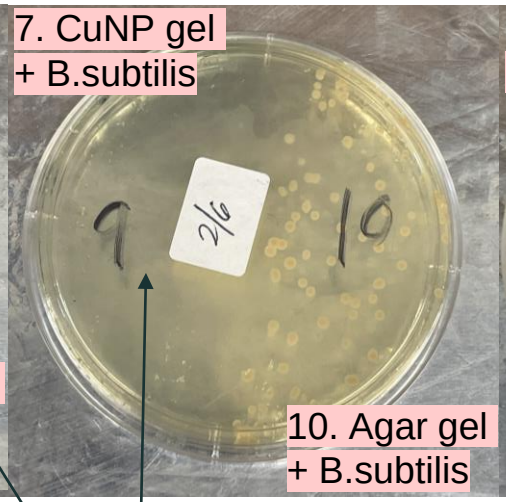
Copper Nanoparticle (CuNP) Results

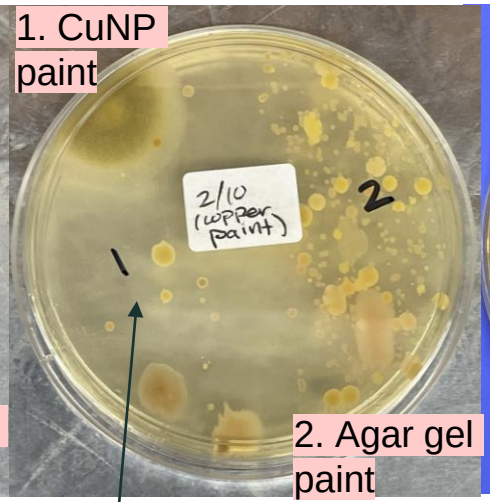
CuNP 25nm				
	Suspension	Gel	Paint	Bandage
	250µL	0.25mg	0.25mg	5µL
Weight mg	5.00E+00	5.00E-03	5.00E-03	1.00E-01
# of Particles	6.84E+16	6.84E+13	6.84E+13	1.37E+15
E.coli	*	*	NA	*
B. subtilis	*	*	NA	*
Bacteria in the air	NA	NA	**	NA

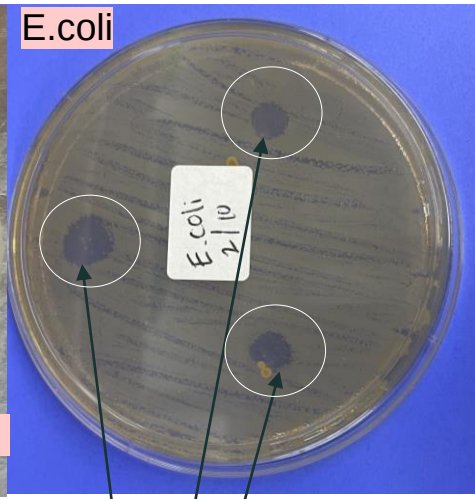
Legend

No bacterial growth	*Less bacterial growth (than negative control)*	*Full bacterial growth (in comparison to negative control)*
*	**	***









Copper Nanoparticle (CuNP) Experiment Results

CuNP Gel (7, 9) shows areas with no bacterial growth (7- E.coli,9- B.subtilis)

Copper Paint (1) shows areas with no bacterial growth

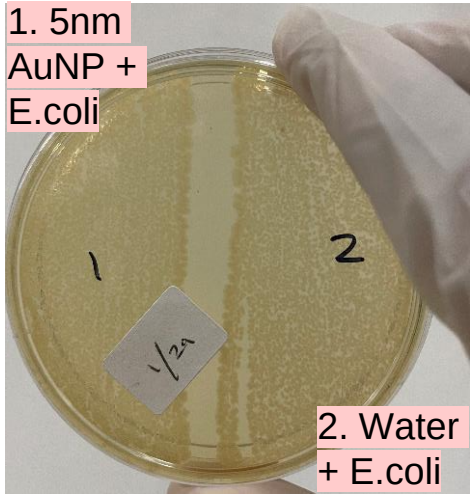
Bandage simulation- shows areas with no bacterial growth in E.coli

Discussion

1) AuNPs were ineffective and did not show bactericidal characteristics:

Possibilities:

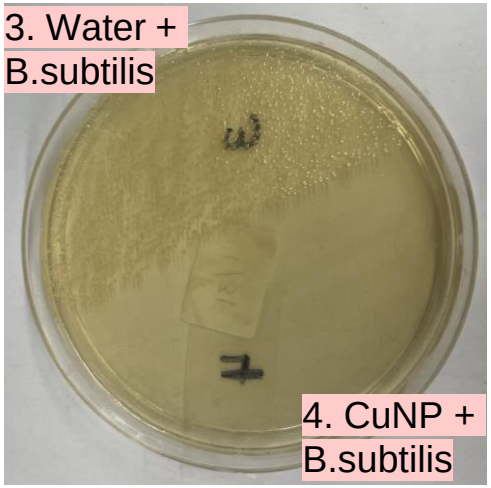
- Initial results showed that AuNPs were ineffective at the concentration used
- AuNP were not ionized or simply too inert by themselves
- AuNP could not diffuse/travel through gel



Results showing AuNPs were ineffective in inhibiting bacterial growth

2) CuNP Paint results were partially successful/ effective:

- CuNP paint applied on paper plate was left in open environment. Bacteria from the air was being captured in the gel for the course of 48 continuous hours meaning copper was exposed to much more bacteria



Results showing CuNPs were effective in inhibiting bacterial growth

3) CuNP demonstrated bactericidal properties:

- Direct Application (in suspension)
- Application as a surface protectant paint
- Application as a gel
- Application as wound dressing

Discussion (Contin.)

A Brief Cost Estimation of Copper Nanoparticle based Wound Dressing

There is high potential to produce CuNP based bandage at a much lower cost than comparative Ag-based product in the market

Description	Quantity	Note
CuNP suspension	20 mg	per mL
4" x 4" bandage	1.25 mL	CuNP suspension applied
CuNP in 4" x 4" bandage	25 mg	Weight of CuNP in mg
Total Copper available	5g	Copper Acquired (Sigma-Aldrich)
Total 4"x4" bandages can be produced	**200**	**5g/25mg**
Cost		
Untreated 4"x4" bandage (cost from internet)	$0.53	https://bodyarmormedical.com/
200 untreated 4"x4" bandage	***$106.00***	200 x 0.53
Copper (purchased for project)	***$70***	5g used for 200 bandages
Other est. expenses of production, shipping, etc	***$170***	Estimated roughly equal to material cost
Total cost estimated for 200 - 4"x4" bandages	***$346.00***	$106+$70+$170
Cost Estimate per 4"x4" CuNP based bandage	**$1.73**	$346/200

Comparable price for Silver based wound 4"x4" product: **$11.47**

https://tinyurl.com/8wa9h6f7

Conclusion

- Through my experiments I concluded:

Copper Nanoparticles (CuNPs) are the **best candidate** for treating pressure sores because of its antibacterial properties, lower cytotoxicity than harmful Silver (Ag), and lower cost than Ag or Gold (Au)

- Gold Nanoparticles (AuNPs) at the concentration used did not reach the Minimum Inhibitory Concentration (MIC) or was too inert for E.coli and B. subtilis and therefore, will be ineffective in combating bacteria that forms on pressure sores and cannot assist in safe healing of wounds

- Copper Nanoparticles (CuNPs) showed bactericidal effects in the concentration and broader applications that it was tested with

- CuNP based Gel can be used in rapidly killing of bacteria or stop the spread of bacterial infection.

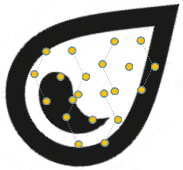
- CuNP based wound dressing has potential as a method of infection control

- CuNP based Gel for antibacterial application on surfaces is effective and may be safe for use on surfaces

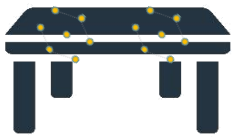
Reflection/Application

- **Through my experiments, I established the high potential applications of Copper Nanoparticle (CuNP) based products.**

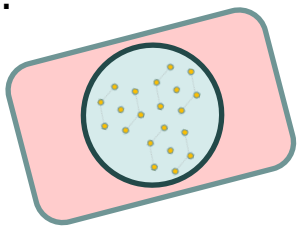
- Next stage of this project will be to establish the quantitative MIC for CuNPs and understanding the mechanism of how CuNP could play a role in anti-biofilm and antimicrobial strategies for effective wound healing.



CuNP based Gel for Topical Application



CuNP based Surface Paint for Medical Equipment/ packaging/ Schools / Grocery Stores



CuNP based wound dressing

All images, icons sourced from Adobe Stock Photo

References Cited

1. Codiţă, I., Caplan, D. M., Drăgulescu, E. C., Lixandru, B. E., Coldea, I. L., Dragomirescu, C. C., Surdu-Bob, C., & Bădulescu, M. (2010). Antimicrobial activity of copper and silver nanofilms on nosocomial bacterial species. *Roumanian archives of microbiology and immunology*, *69*(4), 204–212.
2. Fierascu, Irina; Fierascu, Ioana C.; Dinu-Pirvu, Cristina E.; Fierascu, Radu C.; Anuta, Valentina; Velescu, Bruno S.; Jinga, Mariana; Jinga, Viorel. 2019. "A Short Overview of Recent Developments on Antimicrobial Coatings Based on Phytosynthesized Metal Nanoparticles" *Coatings* 9, no. 12: 787. https://doi.org/10.3390/coatings9120787
3. Food and Drug Administration (FDA) Executive Summary, Classification of Wound Dressings Combined with Drugs, Prepared for the Meeting of the General and Plastic Surgery Devices Advisory Panel, September 20-21, 2016, https://www.fda.gov/media/100005/download
4. Ostaszewska, T., Śliwiński, J., Kamaszewski, M., Sysa, P., & Chojnacki, M. (2018). Cytotoxicity of silver and copper nanoparticles on rainbow trout (Oncorhynchus mykiss) hepatocytes. *Environmental science and pollution research international*, *25*(1), 908–915. https://doi.org/10.1007/s11356-017-0494-0
5. Sen, Chandan, K., Gordillo, Gayle M., Roy, Sashwati., Kirsner, Robert., Lambert, Lynn., Hunt Thomas K., Gottrup, Finn, Gurtner, Geoffrey C., Longaker, Michael T. Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy, Wound Repair Regen. Author manuscript; available in PMC 2010 Nov 1.
6. The National Academies Committee on Copper In Drinking Water Board On Environmental Studies And Toxicology Commission On Life Sciences Other Reports Of The Board On Environmental Studies And Toxicology, https://www.ncbi.nlm.nih.gov/books/NBK225407/